

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Corvi Mora et al.	)	Examiner:
			)	Aaron J. Kosar
Serial No	:	10/537,190	)	
			)	Art Unit:
Cnfrm No	:	1281	)	1651
			)	
Filed	:	December 2, 2003	)	
			)	
For	:	QUATERNARY COMPOUNDS	)	
		COMPRISING PROPOLIS AS THE	)	
		ACTIVE SUBSTANCE	)	
			)	

**DECLARATION OF TIZIANA CANAL  
UNDER 37 CFR § 1.132**

**Mail Stop AF**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, Tiziana Canal, declare:

1. I received a University degree (PhD equivalent) in Pharmaceutical Chemistry and Technologies with distinction from University of Trieste, Trieste (Italy) in 1986. I am a the joint inventor of the above-identified patent application.

2. The attached report was written by me and the experiments were conducted under my direction.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Date:03.02.2009

Tiziana Canal

**REPORT ON**  
**US Application 10/537,190**  
**"QUATERNARY COMPOUNDS COMPRISING**  
**PROPOLIS AS THE ACTIVE SUBSTANCE"**

**Applicant: Actimex s.r.l.**

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*Attachment: analytical specifications of Propoflavis and C-Propoflavis*

Report written by

Tiziana Canal, PhD

Actimex



## **1 PURPOSE**

Purpose of this report is answer to some issue raised in the Detailed Action as per August 13, 2008 and to supply a comparison between the a product obtained according to the present invention by grinding of quaternary compositions consisting of propolis, as active substance, a hydrophilic carrier, and two co-grinding auxiliary substance which one is an amino acid and the second is glycrrhizate with a product obtained by grinding of quaternary compositions consisting of propolis, as active substance, a hydrophilic carrier, and two co-grinding auxiliary substance which one is an amino acid and the second is trehalose.

The Examiner objection is that ammonium glychirryzate exhibit such similarities with another substance, namely trehalose, that the use of the first does not add any novelty to the prior art; moreover, since both are soluble in water, they will be both expected to contribute to the solubility of the composition.

## **2 CLARIFICATION OF THE CORRELATION BETWEEN PROPOLIS AND C-PROPOFLAVIS**

The Examiner requires a clarification, an appropriate correlation between propolis and propoflavis.

We shall first review the statements reported in the present invention: first, under "State of the art", it is stated that "...*propolis is a special wax produced by bees which is widely used in phytotherapy as a dietary supplement, .....*"

Further on it is said that : "...*the best raw material however is considered to be the dried extract which is titred in total flavonoids expressed as galangin.*"

Finally, under "Summary of the invention" the following statements could be found: "*The Applicant has now surprisingly found ...compositions in the form of finely divided dried powders, characterised by being quaternary and by comprising propolis as the active substance*" ...thus "...*the object of the present invention are said quaternary compositions, comprising propolis as the active substance, ....*"

For the purpose of a better clarification we could thus say here that:

Propolis is a natural product of bees; it is naturally found in the form of a wax, containing a number of various substances. This is the so called Raw propolis.

This raw material needs to be purified before using, in order to separate the beneficial substances from the unwanted ones (waxes, resins and so on): the resulting product is the Dried extract.

The highly purified dried extract used in the examples of the present invention has been identified by its registered name, Propoflavis®. The analytical specification of a sample of this raw material is herein attached.

The specific compositions cited in Example 1 of the present invention has been identified in the working examples from 5 to 13 with the name of c-Propoflavis (always referring to Example 1), where "c" stands for "composite (material)", in order to clearly distinguish it from the starting dried extract, Propoflavis.

Thus, c-Propoflavis univocally identifies the composition described in Example 1 of the present invention, that is multicomposite material composed by Propoflavis, ammonium glycyrrhizate, β-cyclodextrin and L-glycine in the ratio of 1:1:7.5:0.5 w/w.

The analytical specifications of a sample of these materials (i.e propoflavis and C-propoflavis) are herein attached.

### **3 COMPARISON BETWEEN THE CO-GROUND QUATERNARY COMPOSITION OF THE PRESENT APPLICATION AND QUATERNARY COMPOSITIONS WITH TREHALOSE SUBSTITUTING GLYCYRRHIZATE**

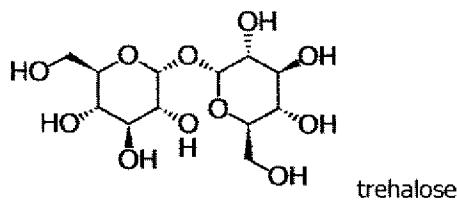
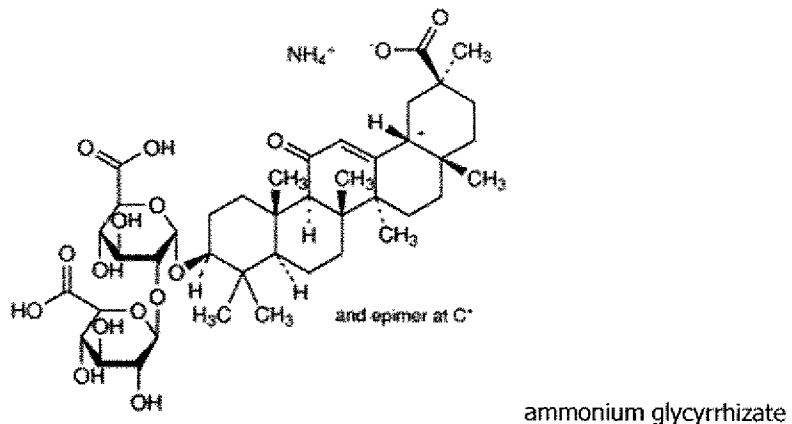
In its claim rejection under 35 USC § 103 (a) as being obvious over Aga (A: PTO-892, 2/1/2007) in view of Mandai (US 6,005,100 A) and Zaffaroni ( US 3,876,816) and Kasori (JP 60188036), the Examiner alleges that "*Mandai, Zaffaroni and Kasori provide motivation for substituting glycyrrhizate and trehalose and Aga further provides the motivation for pulverizing (grinding) the powdery composition.....*" being "*both glycyrrhizate and trehalose ...very soluble in water and thus would be expected to contribute to intrinsic solubility of the bulk composition (i.e. compositions comprising the compounds), including ground compositions thereof*".

Futher on the Examiner argues that "... *the providing a very water-soluble compound /e.g. glycyrrhizate, trehalose) to a composition having a degree of water-solubility, to the contrary, would be expected to positively affect/increase water-solubility of the bulk composition. Also, as argued supra, said property of solubility is an aqueous property and thus compositions having similar chemical combinations and physical (powdered) form, would be expected to intrinsically have the claimed properties, especially in the absence of evidence to the contrary, side by side comparison(s), or evidence to the criticality of some undisclosed features.*"

The assumption for obviousness of the Examiner is based on the facts that:

- i) having both glycyrrhizate and trehalose water-soluble property can be equivalent and used indifferently for the purpose of the invention; and
- ii) the quaternary compositions consisting of propolis, as active substance, a hydrophilic carrier, and two co-grinding auxiliary substance which one is an amino acid and the second is **glycyrrhizate** and that quaternary compositions consisting of propolis, as active substance, a hydrophilic carrier, and two co-grinding auxiliary substance which one is an amino acid and the second is **trehalose** are **similar chemical combinations**.

We respectfully object that in its argument the Examiner does not take in due account the fact that the chemical nature of the substances at issue (see below the molecular formula of glycyrrhizate and trehalose) is not so similar but rather different, thus, the quaternary compositions including glycyrrhizate and trehalose are not similar chemical combinations.



Moreover, we respectfully object that the addition of a soluble component to a poorly soluble mixture of powders is not adding solubility properties to the mixture itself; actually, the different physico-chemical properties of the two powders more easily bring to powders physical separation than to a help in dissolution. The more soluble powder will quickly dissolve while the less soluble will stay on the liquid surface layer or sink in the bottom without dissolving.

Only if materials are brought to an intimate, supramolecular contact through the application of co-grinding process as disclosed in the application the synergistic effect induced by the application of energy will be seen. Actually, during the experimental and scientific work performed in order to find the most suitable quaternary combination, we explored several possibilities and the surprising and final finding was that only ammonium glycyrrhizate was performing as a solubility enhancer.

In order to prove what said above, we are reporting some more experimental findings; for the sake of a better reading we report examples 1 and 2 of the present invention as well as examples 3 and 4 (ternary compositions for comparison respectively with 1 and 2); moreover, we add 2 more examples (coded as examples 5 and 6) with trehalose substituted to glycyrrhizate and treated under the same experimental conditions.

#### **Example 1 (from present application)**

#### **Quaternary Composition with Propolis, Ammonium Glycyrrhizate, $\beta$ Cyclodextrin and L-glycine**

2 Kg of a propoflavis, ammonium glycyrrhizate,  $\beta$  cyclodextrin and L-glycine mixture, in the ratio of 1:1:7.5:0.5 w/w, are homogenised for 10 minutes in a rotating body powder mixer. The mixture is loaded

into a vibrational mill equipped with sintered alumina cylindrical milling means and subjected to grinding with a vibrational amplitude comprised of between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 98.9%, is sieved and 99.8% of the product recovered in the form of a finely divided, free-flowing powder.

### **Example 2 (from present application)**

#### **Quaternary Composition with Propolis, Ammonium Glycrrhizate, β Cyclodextrin and Glutamic Acid**

2 Kg of propoflavis, ammonium glycrrhizate,  $\beta$  cyclodextrin and glutamic acid mixture in a ratio of 1:1:7.5:0.5 w/w, are homogenised per 10 minutes in a rotating body powder mixer. The mixture is loaded into a vibrational mill equipped with sintered alumina cylindrical milling means and subjected to grinding with a vibrational amplitude comprised of between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 98.9%, is sieved and 99.8% of product recovered in the form of a finely divided free-flowing powder.

The quaternary compositions described may be replicated using, as the aminoacid co-grinding auxiliary, substance, other aminoacids amongst which are lysine and serine.

### **Example 3 (from present application)**

#### **Ternary Composition with Propolis, β Cyclodextrin and L-glycine**

1 Kg of propoflavis,  $\beta$  cyclodextrin and L-glycine mixture in a ratio of 1:7.5:0.5 w/w, are homogenised for 10 minutes in a rotating body powder mixer. The mixture is loaded into a vibrational mill equipped with sintered alumina cylindrical milling means and subjected to grinding with a vibrational amplitude comprised of between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 97.8%, is sieved and 99.6% of product recovered in the form of a finely divided free-flowing powder.

### **Example 4 (from present application)**

#### **Ternary Composition with Propolis, β Cyclodextrin and Glutamic Acid**

1 Kg of propoflavis,  $\beta$  cyclodextrin and glutamic acid mixture in a ratio of 1:7.5:0.5 w/w, are homogenised for 10 minutes in a rotating body powder mixer. The mixture is loaded into a vibrational mill equipped with sintered alumina cylindrical milling means and subjected to grinding with a vibrational amplitude comprised of between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 97.8%, is sieved and 99.6% of product recovered in the form of a finely divided free-flowing powder.

### **NEW example 5**

#### **Quaternary Composition with Propolis, Trehalose, β Cyclodextrin and L-glycine**

1 kg of propoflavis, trehalose,  $\beta$  cyclodextrin and L-glycine mixture, in the ratio 1:1:7.5:0.5 w/w, are homogenised for 10 minutes in a rotating body powder mixer. The mixture is loaded into a vibrational mill

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equipped with sintered alumina cylindrical milling media and subjected to grinding with a vibrational amplitude comprised between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 98.6% is sieved and 99.7% of the product recovered in the form of a finely divided free flowing powder.

#### **NEW example 6**

##### **Quaternary Composition with Propolis, Trehalose, $\beta$ Cyclodextrin and Glutamic acid**

1 kg of propoflavis, threalose,  $\beta$  cyclodextrin and glutamic acid mixture, in the ratio 1:1:7.5:0.5 w/w, are homogenised for 10 minutes in a rotating body powder mixer. The mixture is loaded into a vibrational mill equipped with sintered alumina cylindrical milling media and subjected to grinding with a vibrational amplitude comprised between 6 and 10 mm for 1 hour.

The product obtained, with a yield of 98.8% is sieved and 99.6% of the product recovered in the form of a finely divided free flowing powder.

Results are reported below in the table, which is an extension of the one reported in the present application.

TABLE 1

Compositions	Solubility	Increase
A-Propoflavis raw material	465 µg/ml	
B-example 3 (ternary w/ glycine)	1695 µg/ml	3.6 fold over A
C-example 1 (quaternary w/ glycine & amm.glyc.)	2430 µg/ml	5.2 fold over A
		1.4 fold over B
New Example 5 (quaternary w/ glycine & trehalose)	1692 µg/ml	Equal to B 1.4 fold less than C
D-example 4 (ternary w/ glut.acid)	1584 µg/ml	3.4 fold over A
E-example 2 (quaternary w/ glut.acid & amm.glyc.)	1999 µg/ml	4.3 fold over A 1.3 fold over D
New Example 6 (quaternary w/ glut.acid & trehalose)	1588 µg/ml	Equal to D 1.3 fold less than E

From the results shown above, it is clearly apparent that the ternary composition containing an aminoacid as ternary co-grinding substance causes a significant improvement in the solubility of Propoflavis, but that with the addition of the second co-grinding substance—ammonium glycyrrhizate—the solubility increases further by a range comprised of between 30 and 40% with respect to the corresponding ternary composition.

It is as well apparent from the comparison above that the grinding of trehalose together with the components of the ternary composition under the same experimental conditions does not add any further improvement to the ternary composition itself, thus showing the novelty added by ammonium glychirrize .

Report written by

Tiziana Canal,PhD

**CERTIFICATE OF ANALYSIS Nr. C-07048****EMISSION DATE** August 29 2007**PRODUCT** c-PROPOFLAVIS®**BATCH** PR04040**MFG. DATE** Sept 2004**EXP. DATE** Sept 2008**ADDITIONAL INFO** Theoretical Propoflavis® content (dry powder): 10 %, corresponding to 2.3 mg of total flavonoides express as Galangin

TYPE OF ANALYSIS	M.U.	SPECIFICATIONS	RESULTS
Product appearance	/	Light brown powder	Conforms
UV Identification	/	Spectra comparison	Conforms
UV Assay (mg total flavonoides express as Galangin / 100 mg dry product)	mg	Not less than 2.2	2.7
Water content (Karl Fischer)	%	Not more than 15.00%	10.8
Microbiological tests	/	Ph. Eu. 5° Ed. 5.1.4. 3B	Not retested

First emission CoA C-05026 in May 2005.

**NOTES** Product retested for chemical and physical properties in August 2007.  
In accordance to last stability results, product c-PROPOFLAVIS® shelf life is extended to 4 years**QC MANAGER****QUALIFIED PERSON**

<b>QUALITY-CONFORMITY CERTIFICATE</b>	
PRODUCT: <b>PROPOFLAVIS®</b>	PRINT DATE 05/06/2004
DESCRIPTION: DRY EXTRACT FROM RAW PROPOLIS	BATCH: BESPC0104/EU
ORIGIN:FRANCE OR ITALY	ANALYSIS:102-1/04 PROD.DATE: NOVEMBER 2004
EXTRACTION SOLVENT: ETHANOL	RETEST DATE: NOVEMBER 2009

**MANUFACTURER: EUPHAR GROUP SRL**

**APPEARANCE: YELLOW BROWN FINE POWDER, ODOUR CHARACTERISTIC.**

PHYSICAL/CHEMICAL TEST	UNITS	LIMITS	RESULT
IDENTIFICATION (TLC)		CONFORMS TO INTERNAL REFERENCES	CONFORMS
LOSS ON DRYING	%	<10%	CONFORMS
RESIDUAL SOLVENT (ETHANOL)	%	≤0.5	CONFORMS

ASSAY	UNITS	LIMITS	RESULT
TOTAL FLAVONOIDS,EXPRESSED AS GALANGIN	%	≥17%	18.23

HEAVY METALS	UNITS	LIMITS	RESULT
Pb	ppm	≤ 3.0	CONFORMS
Cd	ppm	≤ 0.5	CONFORMS
Hg	ppm	≤ 0.3	CONFORMS

RADIOACTIVITY	UNITS	LIMITS	RESULT
RADIOACTIVITY	Bq/Kg	≤ 600	CONFORMS

  
**EUPHAR** GROUP  
*Bioagrindustria*

MICROBIOLOGY	UNITS	LIMITS	RESULT
AEROBIC BACTERIA	CFU/g	≤ 10000	≤ 10
FUNGI	CFU/g	≤ 100	≤ 10
ENTEROBACTERIACEAE	CFU/g	≤ 100	≤ 10
ESCHERICHIA COLI	CFU/g	ABSENT IN 1g	ABSENT
SALMONELLA	CFU/g	ABSENT IN 10g	ABSENT

PESTICIDES	RESULT
According to list of European Legisl. and organic/biological statement	CONFORMS

ANTIBIOTICS	RESULT	
TETRACYCLINES AND ANALOGUES	According to organic/biological Statement	ABSENT
CHLORAMPHENICOL AND ANALOGUES		ABSENT
SULPHATYAZOLES AND ANALOGUES		ABSENT

OTHER INFORMATIONS	RESULT
IRRADIATION	According to organic/biological Statement
GMO	NOT IRRADIATED
ALLERGENS	According to Allergen Statement
	FREE
	CONFORMS

BIOLOGICAL CONFORMITY-	
MANUFACTURER BIOLOGICAL CERTIFICATE N°IT ICA H613	

FINAL JUDGEMENT		
APPROVED	√	QC: Dr. Elena Mencini

## DATA SHEET

***c-Propoflavis®***

<i>Description</i>	<p>c-Propoflavis® is a multicomposite of  <b>Propoflavis® / β-cyclodextrin / Ammonium Glycyrrhizate / Glycine</b>          in a w/w ratio  <b>10 / 75 / 10 / 5</b></p>				
		<i>Propoflavis</i>	<i>β-Cyclodextrin</i>	<i>Ammonium Glycyrrhizate</i>	<i>L- Glycine</i>
	<i>Molecular Formula</i>	not applicable	C <sub>42</sub> H <sub>70</sub> O <sub>35</sub>	C <sub>42</sub> H <sub>55</sub> NO <sub>16</sub>	C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub>
	<i>Chemical Name</i>	not applicable; it is a purified extract of Propolis, with a min content of flavonoids expressed as Galangin 22 %	Cyclo heptaamyllose	3-O-(2-O-β-D-Glucopyranuronosyl-α-D-glucopyranuronosyl)-18β-glycyrrhetic acid, ammonium salt	Amino ethanoic acid
	<i>INCI Name</i>	Propolis	Cyclodextrin	Ammonium Glycyrrhizate	Glycine
	<i>Cas N.</i>	not applicable	7585-39-9	53956-04-0	56-40-6
	<i>Mol. Weight</i>	not applicable	1134.98	840.0	75.07
<i>Appearance</i>	Amber to light yellow powder				
<i>Odour</i>	Sharp characteristic odour				
<i>Solubility</i>	≥ 2 mg/ml in pH 6.8 buffer at 37°C				
<i>Specification</i>	<i>Appearance</i>		Amber to light yellow powder		
	<i>Smell</i>		Sharp characteristic odour		
	<i>Identity (UV)</i>		Conforms as reference spectrum in pH6.8/ Ethanol 60/40 v/v with maximun at 290 ±3 nm and 323±3 nm		
	<i>Assay (UV)</i>		Total flavonoids as Galangin content in 100 mg of dry powder: 2.2-3.0 mg of total flavonoids as Galangin (according to Propoflavis® % content of total flavonoids as Galangin 26±4 )		
	<i>UV Specific Extinction (E1%,1cm, pH 6.8)</i>		657.7 ± 10 ( $\lambda = 353 \pm 2$ nm)		
	<i>Water content</i>		not more than 15.0%		

<u>Applications</u>	c-Propoflavis® is a multicomposite resulting by the application of the mechano-chemical activation technology to a purified extract of Propolis named Propoflavis®. The technology improves Propoflavis® antibacterial and antiviral properties: solubility is increased of about 50 times and bioavailability of about 40 times with respect to a standard Propoflavis® dry extract. It may be used in the formulation of food supplements to be used as adjuvants in the treatment of e.g. oral cavity infections (e.g. tablets, capsules, sachets, sprays, syrups) or in cosmetic products (e.g., oral sprays, labial creams, lipsticks, milks, oral rinses lotions,).
<u>Dosage</u>	Mechano-chemical activation does not modify the properties of the molecule neither changes its chemical structure. Therefore the recommended dosage are those necessary to achieve the desired effect according to formulation – keeping into account that the content in Propoflavis d.e. is 10% corresponding to 2.6 % total flavonoids as Galangin. Orientative dosages: oral dosage forms: up to 50 mg/dose. Creams: 2-4% content. Sprays, solutions: 2-5%. Maximum oral daily dose: 350 mg/day
<u>Stability</u>	Shelf life for c-Propoflavis® is four years in an originally sealed container Store in a dry place at a temperature below 30°C. Protect against light.
<u>MSDS</u>	A safety data sheet is available
<u>Note</u>	The data submitted in this publication are based on our current knowledge and experience. They do not constitute a guarantee in the legal sense of the term and, in view of the manifolds factors that may affect processing and application, do not relieve processors from the responsibility of carrying their own tests and experiments. Any relevant patent rights and existing legislation and regulations must be observed.